The Origin, Development, and Nature of the Spermatophoric Mass of the Spiny Lobster, *Panulirus penicillatus* (Oliver)[1]

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INTRODUCTION

ALTHOUGH MANY thorough investigations of crustacean spermatozoa have been made, few have included more than desultory accounts of the spermatophores. The material presented in this paper not only extends our knowledge of the biology of the spiny lobster *Panulirus penicillatus* (Oliver) through an account of the origin, development, and nature of its spermatophoric mass, but also suggests the method by which its spermatozoa are liberated.

Early cursory observations on crustacean spermatophores by Cavolini, Kölliker, and Schwammerdam, cited in Herrick (1895: 160), apparently failed to stimulate further interest in this direction, but possibly served to incite such workers as Grobben (1878), Hermann (1890), Sabatier (1893), Brandes (1897), Labbé (1903), and Koltzoff (1906) to investigate more thoroughly the nature of decapod spermatozoa. These early works illustrate the interest in the spectacular decapod spermatozoa to the neglect of the spermatophores.

With the exception of the early work of Herrick (*op. cit.*) on the reproductive system of the American lobster (*Homarus americanus*), investigations of the spermatophores of macrurans were singularly lacking until Dahlgren and Kepner (1908) attributed to the lobster and the crayfish a fluid which, secreted by the walls of their sperm ducts, not only served as a vehicle to carry the mass of sperm but also formed a semifluid covering around them. Dahlgren and Kepner further reported that the spermatophore attached itself to a receiving plate on the female, became hard, and preserved the life of the spermatozoa for months or even years. No reference is made however to the species of lobster and crayfish which produced these spermatophores.

No particular differentiation between the non-pedunculate spermatophores of the Macrura and the more bizarre pedunculate spermatophores of the Anomura and Brachyura was noted in the literature until Calman's treatise in 1909. Since that time considerable research has ensued on the pedunculate type, and, culminating in the studies of Mouchet (1930–31), the origin and development of these have become rather well known.

Comparatively little research has been accomplished on the non-pedunculate spermatophore of the Macrura. Allen (1916) first described for a spiny lobster, *Panulirus interruptus*, a spermatophore composed of a putty-like mass of sperm material. This mass, placed on the ventral surface of the female's thorax, contained contorted tubular cavities filled with spermatozoa. He further explained that this mass was at first white and soft but later turned black and became hard, resembling whalebone. Allen's account, as well as Fasten's (1917), on the consistency and color change in the spermatophoric mass of *Panulirus interruptus* is in accord with the findings of this report, but their observations on the contorted tubular cavities were not extensive and little consideration was given to the origin or the development of the mass. Insofar as can be ascertained, no published work on the origin, development, and nature of the spermatophoric mass of *P. penicillatus* has been reported.

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METHODS AND TECHNIQUES

Specimens of *P. penicillatus* taken in the vicinity of Kaneohe Bay, Oahu, between July, 1947, and January, 1948, were used in this study. Males whose carapace length exceeded 10 centimeters were usually found to possess well-developed gonads. These were removed from freshly killed specimens, rolled lightly on blotting paper, weighed, and placed immediately in fixative. A label indicating the catch and specimen number was inserted in each vial so that histological data could be correlated with size, date, and other pertinent information.

For the purpose of routine histological examination the reproductive system (Figs. 1, 2) was divided into: anterior testis (Fig. 2*i*); mid-testis (*e*); posterior testis (*c*); proximal vas deferens (*d*); and the massive distal vas deferens (*h*). Small portions of these regions were placed in Bouin's fixative, which gave excellent preparations for all tissue except the vas deferens which became extremely brittle. Tissues were cleared with toluene, embedded in Tissuemat (54–56° C.) and, for routine investigation, sectioned at 10 microns. Some testis preparations were placed in Bouin's fixative and some in Fleming's, and were cut at 6 and 4 microns. Routine sections were stained with standard alum-haematoxylin and counterstained with eosin (0.5 per cent solution in 90 per cent alcohol to which 04. cc. of 0.1 N HCl was added). Heidenhain's iron-haematoxylin without a counterstain was used with good results in some sections of the testis. Mallory's triple connective-tissue stain was employed to aid in tissue differentiation. Because of the extreme hardness of the enlarged portions of the vas deferens, it was often necessary to coat the surface of the paraffin block with thin celloidin between successive cuts. For examination of the rayed spermatozoa, fresh sections were taken from the enlarged vas deferens, the matrix was separated from the spermatophore, and the spermatophore was opened in sea water.

Figures 1 and 9 were drawn by Florence Lambeth from dissections made by the author. Figures 2, 4, 5, 6, 7, and 8 were drawn by Inger Achton from slides and dissections made by the author. Figure 3 was drawn by Evan L. Gillespie.

The writer acknowledges with thanks the help of Dr. Robert W. Hiatt, who suggested the problem and offered his assistance in many ways throughout the course of the study.

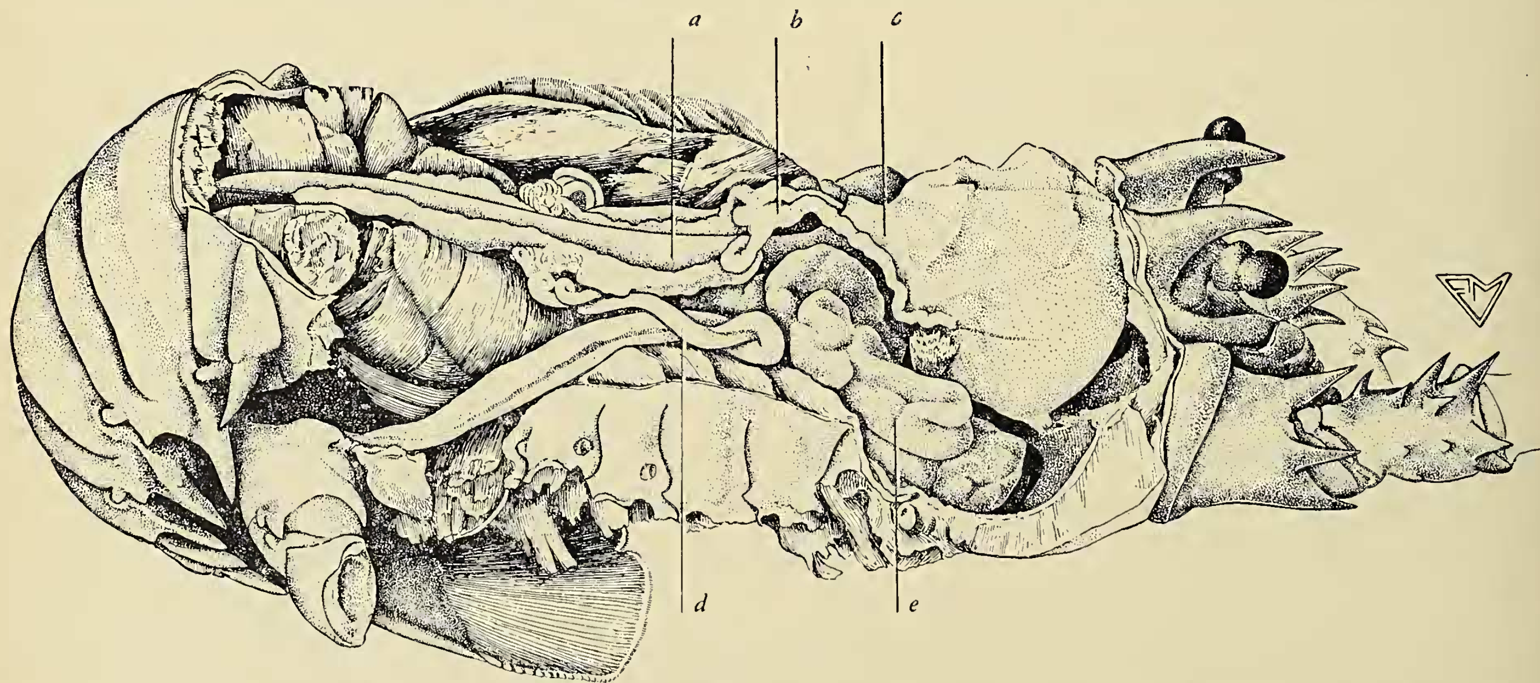

FIG. 1. Dissection of a mature male *Panulirus penicillatus* (Oliver) drawn to show the structure and position of the reproductive system in relation to other structures. *a*, Intestine; *b*, testis just anterior to transverse bridge; *c*, pyloric region of stomach; *d*, enlarged portion of vas deferens; *e*, hepatopancreas. (0.5×)

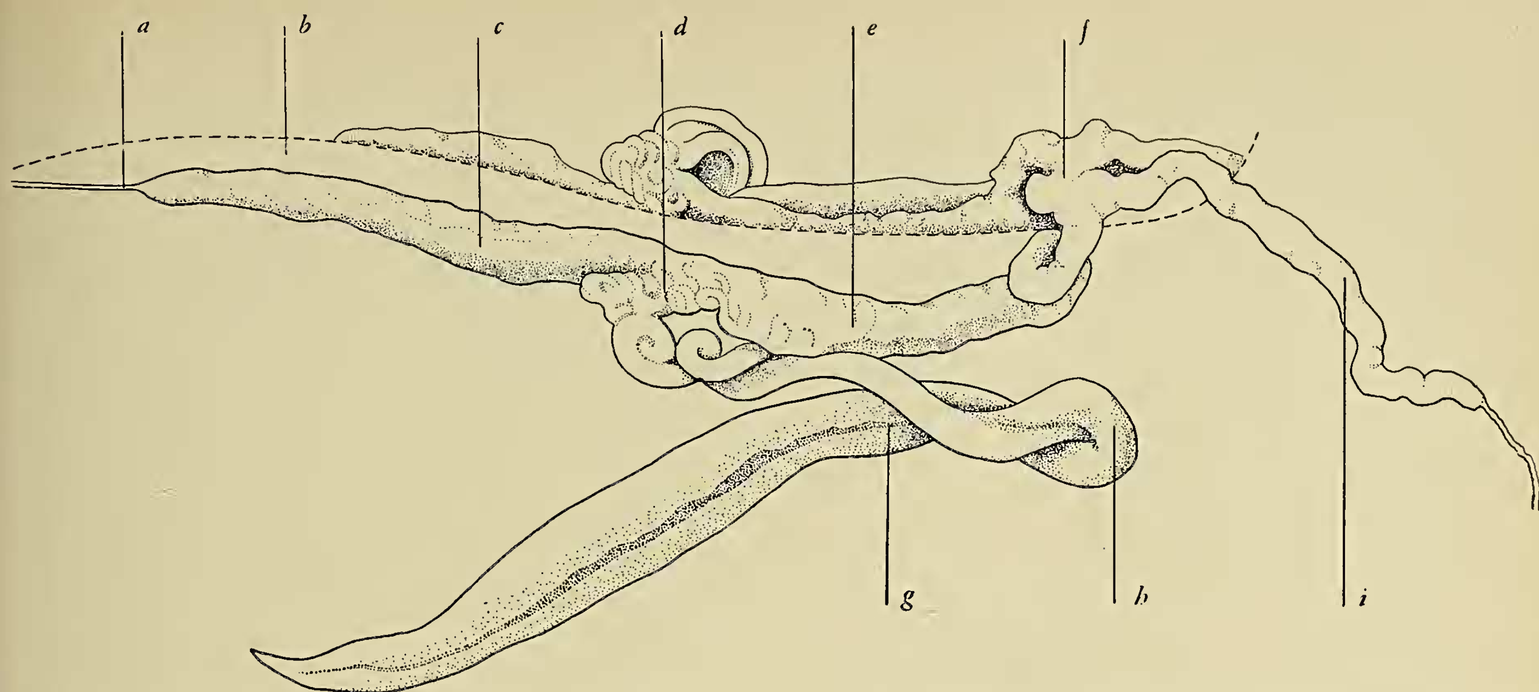

FIG. 2. Male reproductive system enlarged to show: *a*, posterior mesentery of testis; *b*, portion of the digestive tract; *c*, posterior region of testis; *d*, proximal portion of vas deferens; *e*, region of mid-testis; *f*, region of transverse bridges; *g*, hyaline line; *h*, enlarged, swollen portion of vas deferens; *i*, anterior region of testis. (1×)

RESULTS

The testes (Fig. 1*b*) are seen to be long, whitish, sacculate tubes joined one to the other by a transverse bridge (Fig. 2*f*) just posterior to the junction of the pyloric region of the stomach (Fig. 1*c*) with the intestine (*a*). Seen dorsally, the testes resemble an elongated **H** in which the posterior portion (Fig. 2*c*) usually exceeds the anterior portion (*i*) both in length and diameter. With the exception of the anterior distal portion of the testis which, as it encircles the stomach, extends ventrally, the major portion of the anterior testis lies dorsal to the hepatopancreas (Fig. 1*e*) and the digestive tract (Fig. 1*a*, 2*b*). The posterior portion of the testis traverses caudad on either side of the intestine, often slightly ventrad to the digestive tract. It is not uncommon to observe differences in the size of the testicular horns, the right posterior portion often being longer than the left, or vice versa. The testes are held in place by mesenteries (Fig. 2*a*) which extend posteriorly a short distance into the first abdominal segment, and anteriorly appear to be attached ventrad to the stomach.

At the outset of this study it became apparent that an understanding of the origin, development, and nature of the spermatophoric mass made necessary a thorough study of the histology of the testis. Only when the entire testis is studied in serial section can one properly interpret any single section or attempt the more difficult task of reconstruction. Throughout each general region of the testis, follicles observed in any one histological section exhibited various degrees of maturity, although the cells in any one follicle were usually at the same stage of development.

Figure 3 is a diagrammatic reconstruction of the testis. It suggests, somewhat, a paniculate inflorescence, but seems to be a racemose or compound gland of freely branching ducts which terminate in acini, so that the whole resembles a compact cluster of grapes. The reconstruction further shows that the racemose, anterior portion of the testis (*a*) joins ultimately the racemose, posterior portion of the testis (*b*) to form an exceedingly long and highly coiled tube (*c*). This contorted tube traverses the testis for some distance both anteriorly and posteriorly from its place of origin in the mid-testis (*d*) and ultimately emerges as the vas deferens (Figs. 1*d*, 2*d*, 3*e*).

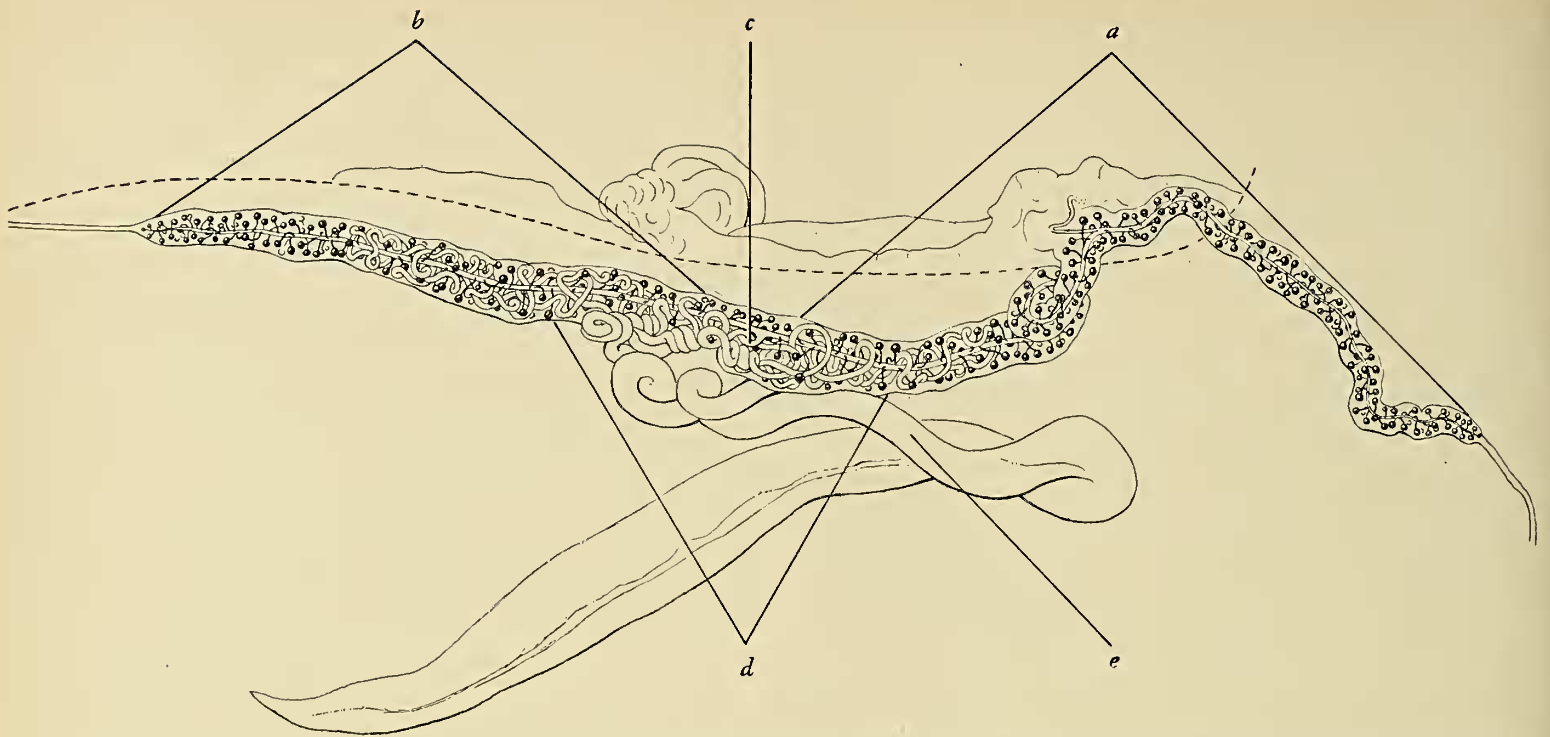

FIG. 3. Diagrammatic reconstruction of the right testis to show: *a*, paniculate anterior portion; *b*, paniculate posterior portion; *c*, tube which ultimately drains regions *a* and *b*; *d*, mid-testis; *e*, vas deferens. (1×)

Figure 4 is a cross section through an immature follicle taken through the anterior part of the testis (Fig. 2*i*). It should be borne in mind, however, that sections taken through the mid-portion of the testis (Fig. 2*e*) or the posterior portion of the testis (*c*) would reveal follicles of similar maturity, and these would also be associated with other follicles of varying degrees of maturity. The entire follicle is filled with large, primary spermatocytes (Fig. 4*b*). These cells measure 15 to 20 microns in diameter and contain nuclei that may attain a diameter of 10 to 14 microns. At this particular stage it is impossible to distinguish the primary spermatocytes from the seminiferous epithelium (Fig. 4*c*).

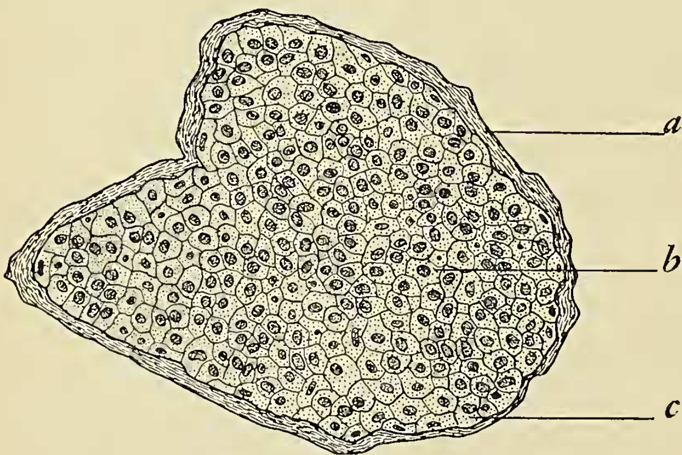

FIG. 4. Camera lucida drawing of portion of testis (Fig. 2*i*). *a*, Connective tissue; *b*, primary spermatocyte; *c*, seminiferous epithelium. (125×)

A transverse section (Fig. 5) as far posterior as the mid-testis (Fig. 2*e*) reveals follicles, some of which are more mature than that illustrated by Figure 4. Follicles at this stage of maturity likewise can be observed in transverse sections throughout the length of the testis, and these would also be associated with other follicles of varying degrees of maturity. A lumen (Fig. 5*g*) and radiating cells (*c*) present a wheel-like appearance in this stage of development. Whereas in Figure 4 the follicle is filled with a homo-

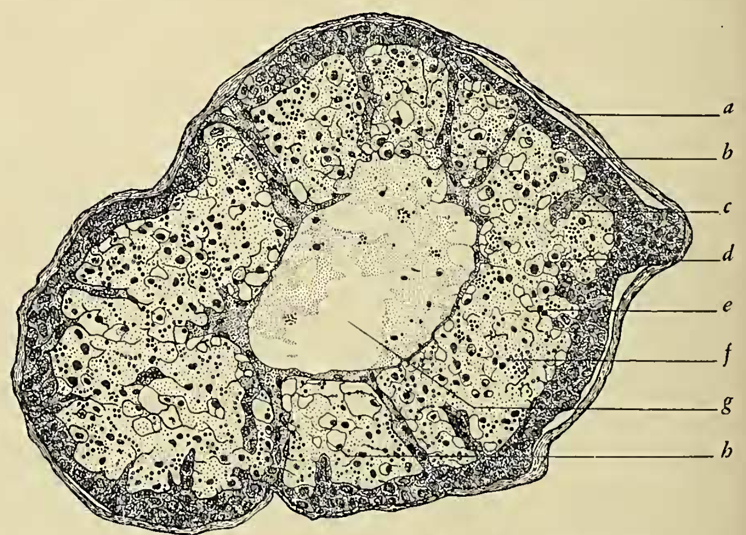

FIG. 5. Camera lucida drawing of a small portion of testis (Fig. 2*e*). *a*, Cumulative tissue; *b*, peripheral layer of epithelium; *c*, Sertoli cells; *d*, primary spermatocytes; *e*, secondary spermatocytes; *f*, spermatids; *g*, central lumen formation; *h*, disintegrating primary spermatocytes. (80×)

geneous mass of primary spermatocytes, in Figure 5 the follicle is filled with a heterogeneous assortment of cells (*d*, *e*, *f*, *h*).

Sections which show the sperm mass in the small collecting tubules are likewise encountered throughout the length of the testis. A longitudinal section of such a tubule (Fig. 6) reveals that a secretion from the epithelium (*b*) also contributes to the continuous sperm mass. Long, fibril-like structures (*c*) are clearly seen, the longitudinal axes of which are parallel to the flow of the sperm mass. The metamorphosing spermatids (*d*) are clumped in rather definite areas and the remainder of the sperm mass (*e*) is scattered indiscriminately. Sections throughout the testis reveal that these tubules coalesce with other similar tubules.

However, sections taken through the regions of the mid-testis (Figs. 2*e*, 3*d*), with the follicles and tubules already referred to, reveal a quite different tube. As indicated by the reconstruction, this tube (Fig. 3*c*) is formed by the juncture of the racemose anterior and posterior portions of the testis, and this highly contorted tube courses throughout a considerable length of the testis (Fig. 3*d*). In many specimens this tube often extends farther posteriorly than anteriorly. Figure 7 represents a cross section through this tube. The greater portion of the epithelium (*f*) is located in glands embedded in the wall of the tube. These glands open into the lumen (*c*) of the tube. The epithelial cells average 43 microns in length and, throughout the entire region, secrete both a mucus-like substance and a granular or crystalline substance (*b*). These "crystals," observed under oil immersion magnification, averaged 1.43 microns in length and were roughly hexagonal. Great masses of these "crystals" (*d*) surround the sperm mass (*e*).

ANATOMY OF THE VAS DEFERENS

Leading from each testis is a highly convoluted tube, the vas deferens (Fig. 1*d*), which,

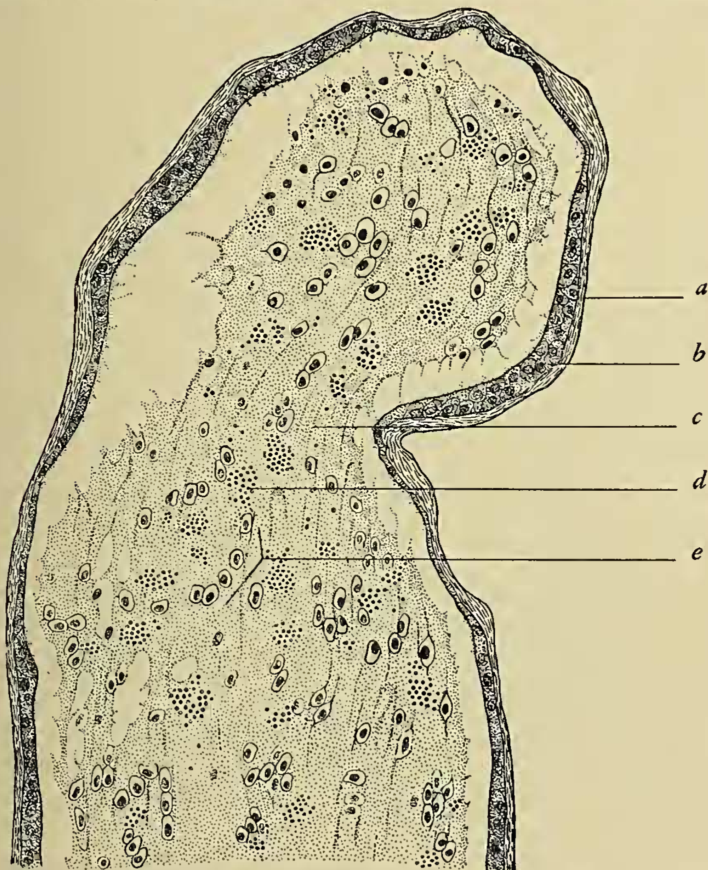

FIG. 6. Camera lucida drawing of a small portion of testis (slightly posterior of Fig. 2*e*) showing tubule in longitudinal section. *a*, Connective tissue; *b*, seminiferous epithelium; *c*, Sertoli fibrils; *d*, developing spermatids; *e*, degenerating primary spermatocytes. (75×)

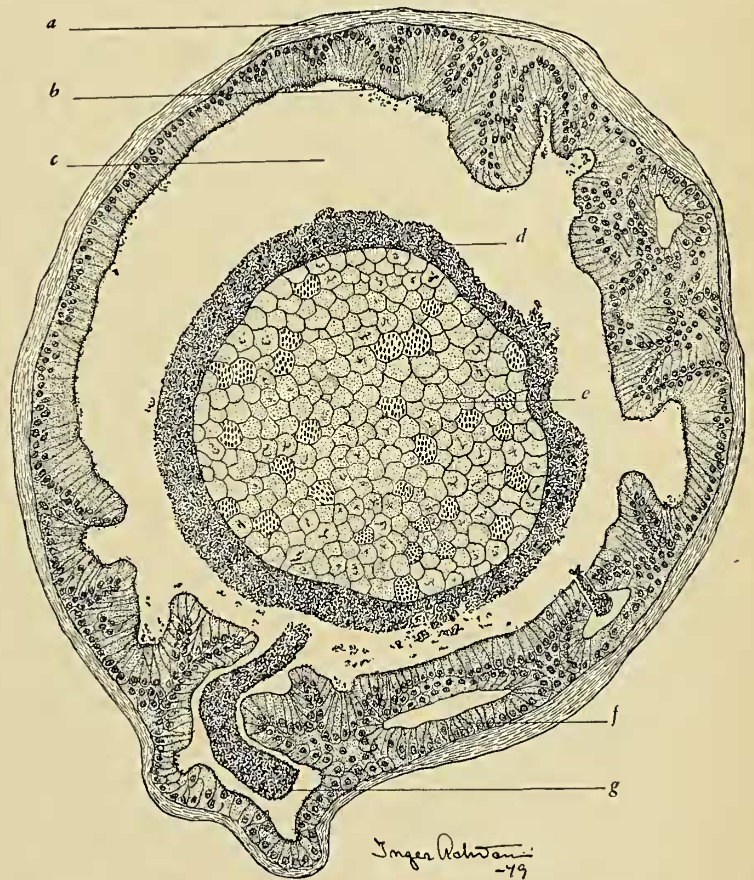

FIG. 7. Camera lucida drawing of a portion of the testis (Fig. 2*c*). *a*, Muscle and underlying connective tissue coats; *b*, crystal-like clumps of material near distal margins of epithelium; *c*, lumen; *d*, wall of spermatophore; *e*, sperm mass; *f*, gland embedded in wall; *g*, spermatophoric wall material exuding from gland. (65×)

increasing in diameter distally, conveys sperm from the testis to the genital pore, located on the coxopodite of the last pereiopod. The proximal portion (Fig. 2*d*), which is impossible to dissect free from the tissue of the testis, emerges to form the enlarged distal portion (Fig. 2*h*). Whereas the testis and certain portions of the vas deferens are opaque, the lateral surface of this enlarged portion of the vas deferens is demarked by a distinct hyaline line (*g*) which traverses its length.

Figure 8 is a typical cross section through the enlarged portion of the vas deferens (Fig. 2*h*). This portion of the vas deferens is characterized by the presence of a typhlosole-like structure (Fig. 8*c*) which projects from the hyaline line (Figs. 2*g*, 8*a*) into the lumen of the vas deferens (Fig. 8*b*). Both the glandular epithelium (*f*) of the "typhlosole," which here bounds deep crypts (*e*), and the connective tissue (*d*) are continuous with the wall of the vas deferens. The wall of the sperm mass (Fig. 7*d*) also appears in sections through the enlarged vas deferens (Fig. 8*g*) but is further embedded in a homogeneous matrix-like substance (*h*). Regardless of the

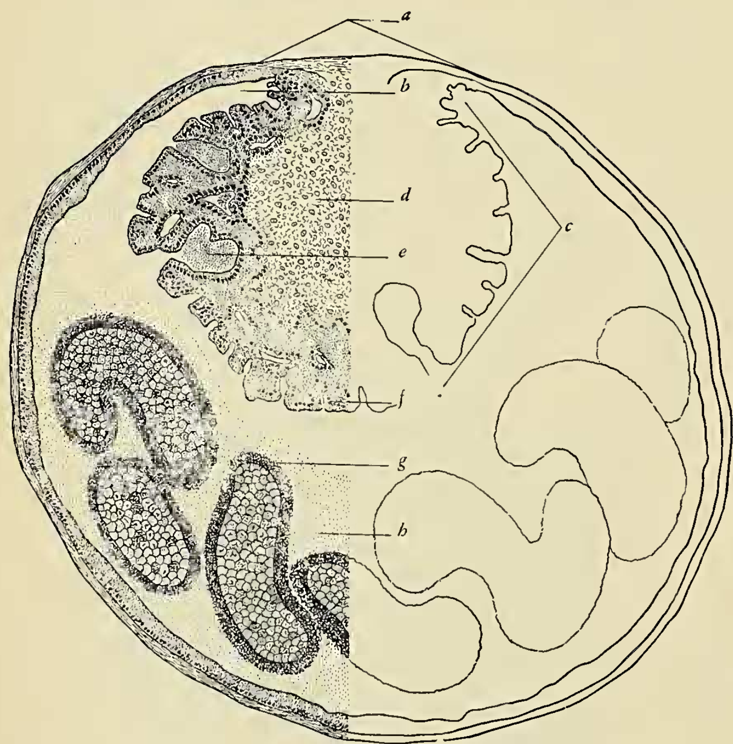

FIG. 8. Camera lucida drawing of a cross section of vas deferens (Fig. 2*h*). *a*, Hyaline line region; *b*, lumen of vas deferens; *c*, "typhlosole"; *d*, connective tissue; *e*, crypt; *f*, glandular epithelium; *g*, spermatophoric wall; *h*, matrix. (13×)

stain used, the glandular secretion (*e*) of the crypt-like folds of the "typhlosole" (*c*) stain the same as the matrix (*h*). Usually, but not in all cases, this secretion takes the cytoplasmic stain.

Dissection of the entire enlarged portion of the vas deferens (Fig. 9) along its lateral line reveals, even prior to flattening and fixation, a highly coiled continuous tube (*a*) embedded in a putty-like matrix (*b*). This tube appears as a yellowish "thread" occupying a position near the muscular wall of the vas deferens opposite the hyaline line.

In freshly killed specimens, peristaltic waves traverse the length of this enlarged portion of the vas deferens when it is pinched with forceps. This stimulation is adequate to cause the extrusion of the spermatophoric mass which in nature adheres to the sternum of the female, posterior to the opening of the genital pore.

Figure 10 illustrates a typical spermatophoric mass on a portion of the sternum (*b*) removed from a female whose carapace length was 9.5 cm. and whose carapace width was 7.6 cm. This specimen was taken off Kekepa Island, Oahu, January 23, 1948, by a tangle net set at 30 feet. The ovaries of this specimen were highly developed and ovulation was imminent. The spermatophoric mass, of about average size, was 4.2 cm. at its greatest width, 2.7 cm. at its greatest length, and approximately 0.5 cm. in thickness. The outer, exposed black portion of the mass (*c*) which could not be peeled off had the consistency of whalebone and averaged less than 0.5 mm. in thickness. As in other specimens, the spermatophoric mass displayed a certain bilateral symmetry suggesting that in its application both right and left vas deferens extrude their contents.

Dissection reveals that the homogeneous inner mass, the matrix (*a*), surrounds the highly coiled, continuous, spermatophoric tube (*d*). Lying under the undisturbed portion of the spermatophoric mass (Fig. 10*c*) is found

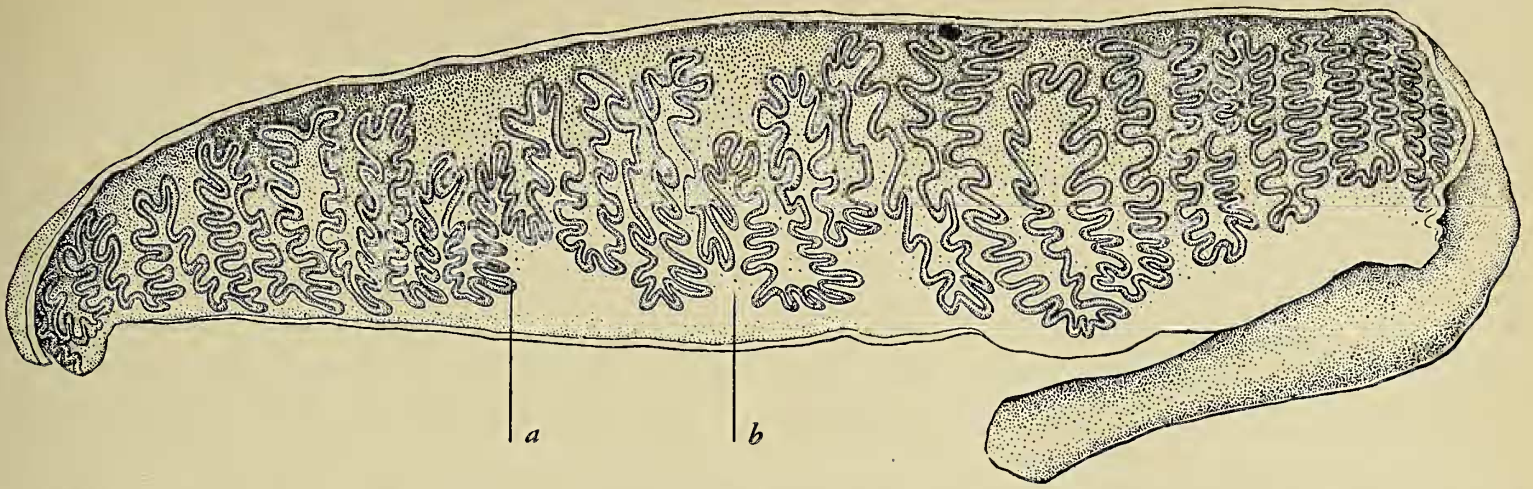

FIG. 9. The enlarged vas deferens dissected along its hyaline line to show: *a*, continuous spermatophore; *b*, region occupied by matrix. (10×)

another continuous spermatophoric tube surrounded by its matrix, products of the opposite or left side of the reproductive system.

DISCUSSION

Since the spermatophoric mass is an accumulation of materials derived from various regions of the male reproductive system, the specific contribution of each region will be discussed in its proper sequence.

By mitotic division, the entire follicle becomes filled with large, primary spermatocytes (Fig. 4*b*). Two processes become evident. From the peripheral layer of the epithelium (Fig. 5*b*) which lies adjacent to the tunica propria (*a*), deeply staining cells (*c*) appear which radiate toward the center of the follicle. At first these are few, some radiating in from one side and some from another. These increase in number without any definite order, creating a wheel-like appearance with the cells forming the "spokes." Care should be exercised in interpreting these early stages because quite often, in sections cut at an angle, these cells appear at the center of the already filled follicle without any relationship to the epithelium from which they arise. Their attached, proximal regions, therefore, would not be evident. Older follicles show these spokes thickened and, at first sight, containing many nuclei. In reality any one spoke is composed of many cells, closely packed together, all radiating toward the center of the follicle. The nuclei, which average 11 microns long and 5 microns wide, are smaller than those of the spermatocyte and more ovoid. These nuclei often appear notched and contain large, deeply staining granules. The reticular, fibrillar nature of the cytoplasm of these cells makes identification of individual cells exceedingly difficult. It should be mentioned that the sections, cut between 5 and 10 microns for routine investigation, are much too thick and too crudely stained for accurate interpretations of the cells. They appear to be Sertoli cells that have actually fused to form a syncytium. This cannot be determined, however, until a more suitable technique is employed.

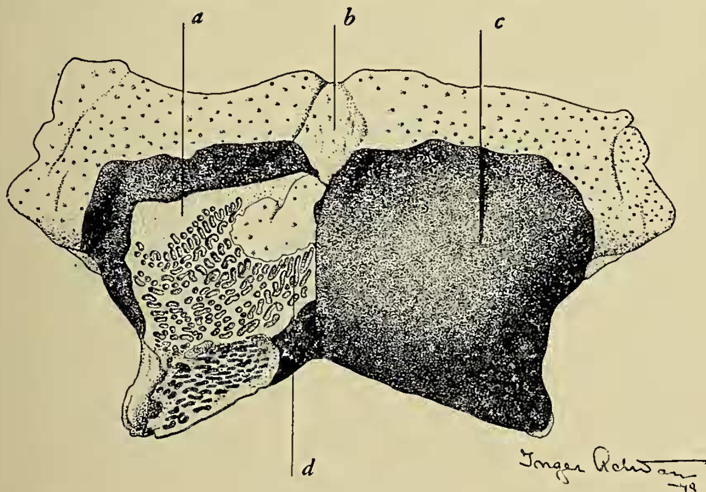

FIG. 10. Spermatophoric mass removed along with a portion of the female's sternum. Right side only dissected to show: *a*, matrix; *b*, sternum; *c*, exposed black portion of mass; d, spermatophoric tube. (1×)

Meanwhile, the second process becomes evident as some of the primary spermatocytes (*d*) undergo two successive divisions, the first

probably heterotypic, resulting in two secondary spermatocytes (*e*), and the second homeotypic, which divides the secondary spermatocytes into four spermatids(*f*). Other potential primary spermatocytes fail to complete the spermatogenic process. In these, small vacuoles appear in their cytoplasm, then coalesce to form a large vacuole which surrounds and often pushes the shrunken nucleus to one side of the cytosome (*h*). All stages of cell disintegration may be observed. The contents of disintegrated cells probably serve as nutrient material both during and after the metamorphosis of the spermatids to spermatozoa. If the central lumen (Fig. 5*g*) that forms in the once compact follicle (Fig. 4) is the result of the action of Sertoli cells, it is possible to assume that what appears to be a lumen bounded by a syncytium of Sertoli cells is in reality the distal portions of many Sertoli cells already containing spermatids. Scattered in between these cells is found the debris of disintegrating primary spermatocytes. In any event, the diameter of the lumen increases and the "ring" of Sertoli cells which surrounds the mass moves toward the periphery of the follicle until, finally, only a single layer of epithelium remains.

A longitudinal section of a collecting tubule (Fig. 6) reveals that the epithelium (*b*) in this region appears to contribute a mucus to the continuous sperm mass. Whether this material serves a nutritive or lubricative function was not determined. Long fibrils (*c*), the longitudinal axes of which lie parallel to the flow of the sperm mass, are clearly seen. It should also be pointed out that, whereas the developing spermatids (*d*) clump in rather definite areas, the remainder of the sperm mass (*e*) is scattered indiscriminately. These tubules, which contain the continuous sperm mass, coalesce with other similar tubules until finally, in the mid-region of the testis (Figs. 3*c*, *d*), sections are encountered which possess structures identical with those shown in Figure 7.

When a comparison is made of sections here illustrated by Figures 6 and 7, the presence or absence of a spermatophoric wall (Fig. 7*d*) is not the only significant difference to be seen. The deposition of this wall (Fig. 7*d*) could possibly account for the compactness of the sperm mass, but could scarcely account for the distinct, cell-like membranes which isolate the developing spermatids into distinct clumps. Whereas the longitudinal sections of the collecting tubule (Fig. 6) present the effect of streaming fibrils (*c*), cross sections through the tube (Fig. 7) clearly show these to be portions of Sertoli cells (*e*) cut at right angles to their longitudinal axes. Whether this isolation into clumps of developing spermatids is due to their having been contained within Sertoli cells that have become liberated, or whether the developing spermatids are mechanically isolated by the pressure of the other cells, is difficult to ascertain. It appears very unlikely, however, that the clumping of the developing spermatids is due to an increase in intercellular pressure because these clusters are first observed in sections of follicles where the lumen (Fig. 5*g*) is just forming, and when the pressure would be negligible. The occurrence of spermatid clusters in the region of initial lumen formation strongly suggests that the spermatids in each cluster represent those individuals that develop within a given Sertoli cell, and that, as the Sertoli cell is sloughed off, the contained developing spermatids remain encompassed.

As previously noted, the wall of the spermatophore (Fig. 7*d*) is encountered when the sperm mass of the collecting tubules (Fig. 6) has reached the position indicated by Figure 3*c*. Throughout the length of this highly coiled tube which traverses the testis for some distance both anteriorly and posteriorly (Fig. 3*d*) the glandular epithelium of its wall secretes the material which surrounds and forms the true wall of the spermatophore. Although the thickness of the spermatophoric wall increases as this portion of the tube is traversed, neither the overall diameter of the

spermatophore nor the thickness of its wall increases once the walled spermatophore enters the enlarged vas deferens. For this reason, together with the fact that the secretion (Fig. 7*g*) from the epithelium of this tube stains identically with the material (*d*), the deposition of the spermatophoric wall is attributed to this tube.

The walled spermatophore (Fig. 7*d*), upon entering the enlarged vas deferens (Fig. 1*d*), becomes surrounded by a putty-like matrix (Fig. 8*h*) which is formed largely by the secretion from the "typhlosole" (Fig. 8*c*). The position of the walled spermatophoric tube (Fig. 8*g*), embedded in the matrix near the wall opposite the "typhlosole," appears explainable by the fact that, although the glandular epithelium of the peripheral wall of the vas deferens may secrete some matrix, the glandular epithelium of the "typhlosole" secretes most of it, and the extensive production of matrix from this region forces the walled spermatophoric tube toward the periphery of the organ. There is no evidence that the wall of the spermatophore (Fig. 8*g*) is formed by this matrix material. Since the hyaline line (Fig. 2*g*) marks externally the position of the internal "typhlosole," it appears hyaline because the dense matrix between the "typhlosole" and the wall of the vas deferens is here lacking.

Although in nature the actual process of extrusion and adherence of the spermatophoric mass was not observed by the author, the process was experimentally performed for subsequent observation. While immersed in sea water, the vas deferens of a mature male *P. penicillatus* was exposed and pinched with forceps. This served as a sufficient stimulus to cause the extrusion of the spermatophoric mass. This mass was smeared on the sternum of a living female, on a piece of sternum which had been removed from an adult female, and on a clean glass plate. The smeared piece of sternum and the smeared glass plate were suspended by threads in the same aquarium with the artificially smeared female. Within the first hour all the smears darkened slightly, but even after 14 days this darkening had progressed only to a light brown color, the masses never attaining the hardness or the darkness of the spermatophoric mass found deposited naturally. The smear on the isolated pieces of sternum and the smear on the glass plate were eagerly sought after and eaten by the reef fish, *Alectis ciliaris* (Bloch). The smears apparently darkened only superficially, for, when viewed through the glass, the unexposed surface could be seen to retain its putty-like appearance. Attempts were also made by this fish to obtain the smear placed on the living female, but she would quickly retreat to a corner of the aquarium or otherwise protect the mass with the telson of her flexed abdomen. During the 14 days of observation this female made no attempt, however, to remove the mass. At the end of this period, the spermatophoric mass was examined carefully and, except for a few superficial sand scratches, the mass was unaltered. The ovaries were then examined and found to be immature. The spermatozoa, however, in the artificially applied spermatophore were still alive. Although this experiment was repeated several times in an attempt to obtain a female in which ovulation was imminent, all efforts failed. In those mature females which obviously had ovulated, the spermatophoric mass was deeply scratched or gouged, and the spermatophores were devoid of spermatozoa.

Andrews (1931), working on the Oregon crayfish (*Potamobius trowbridgei*), was concerned with the manner of liberation of the spermatozoa and stated: ". . . the suggestion was made by Leuckart that by the gradual change of the wall of the spermatophore, (a secretion from the lining cells of the deferent duct), a discharge of sperms might be caused; but this would need be accurately timed. Very likely, as surmised by Meyer, it is the action of some secretion of the female at the time of laying eggs which induces the emptying of the spermatophore." Andrews

mentioned that the spermatophores exhibited free, distinct ends, and their bases were stuck together more or less indistinguishably. The fact that Andrews observed spermatophores over a widely scattered area on the female might possibly be due to the inability of the male to hold fast the female. Since only one case was cited by Andrews, the question arises as to whether this scattering of the spermatophores is the normal manner of application. No mention was made regarding the origin or nature of the adhesive substance, but careful examination of many testis sections of *Potamobius* sp. (prepared commercially by Albert E. Galigher, Inc., Berkeley, California) often reveals a continuous sperm mass enveloped by a spermatophoric wall. Since the developing spermatophore from each testis is in reality a continuous tube, it is possible that the spermatophore in the exuded spermatophoric mass might also be a continuous tube (or two tubes, if each vas deferens contributed simultaneously), as has been shown in *P. penicillatus*. Hurried, intermittent expulsions of the spermatophoric mass by contractions of the muscular vas deferens could account not only for the scattered spermatophoric masses, but also for the formation of the continuous spermatophoric tube into what appears to be separate, distinct spermatophores.

Both Andrews, working with an astacid, the Oregon crayfish, and Herrick (1895), working with the American lobster, a homarid, believe that fertilization is external. On the other hand, von Bonde (1936), working with a palinurid, the Cape crayfish *Jasus lalandii* (Milne Edwards) Ortman, believes fertilization is internal. He describes the condition in *Jasus lalandii* in which the receptaculum seminis of *Homarus americanus* is absent, its counterpart in the position of this structure being only a shallow depression. He further states that this depression is almost covered with hard setae in the living animal and only appears evident in dried specimens, a feature which, he believes, would preclude its use as a receptaculum seminis. The lack of a seminal receptaculum in *Jasus lalandii*, together with the fact that its eggs, when laid, possess an outer membrane of chitin, seem to von Bonde to preclude the possibility of external fertilization and to require that fertilization take place in the oviduct before the chitinous layer is deposited. Moreover, in observations of females after mating and before egg laying, he saw no sign of spermatic fluid on the sternum. Von Bonde concurs with Yonge (1938) that fertilization takes place at the proximal end of the oviduct, and that no difficulty would be encountered by the sperm in achieving fertilization inasmuch as it would need only to penetrate the thin chorion of the egg rather than an outer membrane of chitin. Von Bonde did not explain how impregnation takes place, but it is evident that it is quite different from the manner described herein for another palinurid, genus *Panulirus*.

Crawford and De Smidt (1923) describe the spermatophoric mass for *Panulirus argus* as ". . . composed of two different substances. One substance, which hardens soon after being deposited upon the sternum of the female, forms the bulk and body of the vesicle, while the other substance remains liquid. This liquid does not harden, since it can be expressed when the surface of the vesicle is scraped away. These two substances . . . are probably separated from each other by the process of hardening of the waxy material around the liquid, the pores being formed in a way analogous to the air bubbles in thick glue, or molten glass. . . ." These interpretations are obviously faulty, as demonstrated by my examination of transverse sections of the vas deferens and of the freshly dissected lower portion of this structure.

Wilson (1948), quoting from the unpublished work of Fry (MS.) on the spiny lobster, *P. interruptus*, states, "The presence of putty on the ventral side of a female's carapace is no evidence of maturity as many im-

mature individuals carry sperm. On the other hand, during the breeding season with the exception of a few who had recently cast their shells, no mature female was found which did not have putty or evidence of its former presence." No attempt was made to investigate the contents of the putty-like masses.

The spermatophoric mass on females of *P. penicillatus* which have extruded their ova is deeply scratched; the gouges exhibit a roughened surface and extend through the black, exposed surface, through the matrix, and through the wall of the spermatophoric tube. Figure 10 shows the left side of the spermatophoric mass intact and without deep gouges. When sectioned (right side), the spermatophore contained motile spermatozoa. It should be recalled that this female would probably have discharged her ova presently. Where evidence of the former spermatophoric mass remained, it was deeply gouged, and the exposed, torn spermatophore of both the right and the left side was empty.

Many theories have been advanced for the liberation of spermatozoa from the spermatophore. That the rosette glands of the female's integument may produce a substance which dissolves the capsules of the spermatophoric wall may hold true for the delicate pedunculate spermatophores of the Anomura and Brachyura, but this method of liberating spermatozoa seems very unlikely for *P. penicillatus*. A chemical reaction that would liberate the spermatozoa would have to be exactly timed so that spermatozoa would be available the instant the ova were released. Even so, such action could scarcely have produced the deep, jagged gouges one observes on the spermatophoric mass of *P. penicillatus*. It seems much more reasonable to assume that the spermatozoa are liberated by the scratching action of the female's pereiopods, a method in accord with the observations of Crawford and De Smidt (1923) on *P. argus* and Smith (1948) on *P. interruptus*.

CONCLUSIONS

The **H**-shaped testis of *Panulirus penicillatus* (Oliver), when reconstructed from serial sections, is found to be a racemose or compound gland of freely branching ducts which terminate in acini. The racemose, anterior portion of the testis joins ultimately the racemose, posterior portion of the testis to form a highly contorted tube. This tube, imbedded in the tissue of the testis, traverses for some distance both anteriorly and posteriorly the region of the mid-testis. Cross sections throughout the entire testis show follicles in various degrees of maturity, although the cells in any one follicle usually exhibit the same stage of development.

The immature follicle becomes filled with potential primary spermatocytes by mitotic division of its seminiferous epithelium. A heterotypic, followed by a homeotypic, division of these cells results in the formation of spermatids. Other potential spermatocytes fail to complete the spermatogenic process, and all stages of disintegration of these cells are observed. Further development within the follicles is attributed to the action of Sertoli cells which, arising from the germinal epithelium, encompass the spermatids. The formation of a lumen, giving the follicle a wheel-like appearance, is attributed to the sloughing off of these Sertoli cells.

Longitudinal sections through the collecting tubules show the sperm mass to be composed of four main components: developing spermatids, disintegrating spermatocytes, a nutrient substance, and portions of sloughed-off Sertoli cells. The evidence of spermatid clusters in the region of initial lumen formation as well as in sections of the collecting tubules strongly suggests that the spermatids in each cluster represent those individuals that develop within a given Sertoli cell.

The continuous sperm mass receives its spermatophoric wall from the tube formed by the juncture of the anterior and posterior racemose portions of the testes. The thickness

of this wall increases as this portion of the tube is traversed, but the diameter of the spermatophoric tube remains constant as the enlarged vas deferens is traversed. There is no evidence that the enlarged vas deferens of *Panulirus penicillatus* contributes to the formation of the spermatophoric wall.

The hyaline line of the enlarged vas deferens is associated with a highly glandular typhlosole-like structure. This "typhlosole" produces the bulk of the homogeneous matrix which surrounds the continuous spermatophoric tube. By contraction of the muscular walls of both vas deferens, the spermatophoric mass is exuded.

The spermatophoric mass placed on the sternum of a mature female exhibits a certain bilateral symmetry showing that both the right and left testes and vasa deferentia contribute in its formation. This mass consists of two main components: a continuous, highly convoluted tube—the spermatophore proper—and a putty-like matrix in which the spermatophore is embedded. The spermatophoric mass artificially placed on the sternum of a female hardens and darkens slightly within an hour, but even after 14 days this darkening progresses only to a light brown color, without attaining the hardness or the darkness of the spermatophoric mass found deposited naturally.

Whenever the spermatophoric mass is found scratched or deeply gouged it proves to be devoid of sperm, whereas, if it is found intact, viable sperm are present. Thus, a mechanical method of scratching or gouging the spermatophoric mass for the liberation of the spermatozoa rather than by chemical dissolution is proposed for *Panulirus penicillatus* (Oliver). This method provides ample sperm during ovulation and precludes the probability of internal fertilization.

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